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Elionurus tylophorus (Poaceae: Paniceae: Andropogoneae), a new species from the Kimberley region of Western Australia

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Abstract

Barrett, M.D. & Handasyde, T. *Elionurus tylophorus* (Poaceae: Paniceae: Andropogoneae), a new species from the Kimberley region of Western Australia. *Nuytsia* 30: 177–194 (2019). A new annual species, *Elionurus tylophorus* M.D.Barrett & T.Handasyde, is described in the genus *Elionurus* Willd. of the grass tribe Andropogoneae. It differs from all previously described *Elionurus* species in having keels of the lower fertile glumes ornamented with projections, these often terminated by minute solitary bristles, rather than regular cilia lacking projections, or with projections terminated by hair-tufts in other species. It differs further from *E. citreus* (R.Br.) Benth. and *E. purpureus* E.J.Thomps., the only other Australian *Elionurus*, in having strongly developed fertile glume nerves, rachilla longer and lacking a wing, and presence of lemmas and anthers in the pedicellate spikelet. *Elionurus tylophorus* is currently known from a single location in the King Leopold Ranges in the central Kimberley region, and has conservation significance. A primary division of *Elionurus* into two clades is hypothesised based on a preliminary chloroplast gene phylogeny and two morphological synapomorphies, presence of tufted glume hairs and oil streaks.

Introduction

The purpose of this paper is to describe a previously unknown species of *Elionurus* Willd. that was recently discovered in tropical Australia. The genus *Elionurus* belongs to the largely tropical Poaceae tribe Andropogoneae Dumort. and contains 16 species (Renvoise 1978; Kellogg 2015; Thompson 2017) distributed through Africa, the Arabian Peninsula, India and the Americas, with two disjunct species, *E. citreus* (R.Br.) Benth. and *E. purpureus* E.J.Thomps., occurring in tropical Australia and Papua New Guinea (Thompson 2017). *Elionurus* is absent from south-east Asia, the closest records to Australia being in the Middle East and India.

Elionurus belongs to the distinctive tribe Andropogoneae of subfamily Panicoideae Link, and shares the dominant tribal characters of paired spikelets (one fertile and sessile, the other pedicellate and usually sterile or staminate), disarticulating rachis and C4 photosynthesis (Kellogg 2015). Most species of Andropogoneae have twisted and hygroscopic lemma awns (possibly an apomorphy for the core Andropogoneae), but *Elionurus* is one of the few Andropogoneae genera to lack lemma awns. Although

molecular phylogenies (e.g. Skendzic *et al.* 2007; Teerawatananon *et al.* 2011) strongly support *Eliomurus* as a member of the Andropogoneae, the relationship of *Eliomurus* within the tribe is uncertain and it is unplaced to subtribe in Kellogg (2015) and treated as Andropogoneae incertae sedis. Soreng *et al.* (2017), in their phylogenetic classification of Poaceae, place *Elionurus* in subtribe Tripsacinae Dumort., but many aberrant species of basal Andropogoneae have not yet been sequenced. It should be noted that a significant proportion of species and genera of tribe Andropogoneae are of allopolyploid origin and are difficult to treat in a hierarchical classification based on the principal of monophyly (Estep *et al.* 2014), but *Elionurus* species have not been investigated for allopolyploid origins.

Thompson (2017) provided a detailed discussion of morphological relationships with other genera and identified the presence of a proximal beak on the sessile spikelet as a key distinguishing character for *Elionurus*, which amongst the candidate close relatives is shared only with *Urelytrum* Hack. Most *Elionurus* species can also be identified to genus by the presence of one or two oil streaks on the lower glumes, but this is lacking in three species (Thompson 2017). Important morphological characters delimiting *Elionurus* from other Andropogoneae genera (following Kellogg 2015; Thompson 2017) are: inflorescences unbranched above final leaf blade, rachis fragmenting at maturity, spikelets paired with one sessile and fertile, the other pedicellate and sterile or male, (lower) glume coriaceous to indurated and thicker than the lemmas, fertile spikelets dorsiventrally compressed, lower glume of sessile spikelet with a bifid apex and two distant keels adjacent to an oil streak (streak absent in three species) and with cilia in lines or tufts along at least part of the keel, lemmas lacking awns, and both paleas absent. Most but not all species of *Elionurus* have two distinctive lobes or awns at the apex of the lower glume.

Elionurus citreus is a perennial (or sometimes annual?) species that occurs in tropical and subtropical Australia from the Kimberley to Queensland and south to New South Wales, and also in southern Papua New Guinea (Simon 1992; Simon & Alfonso 2011–). *Elionurus citreus* was considered the only Australian representative of the genus until Thompson (2017) described *E. purpureus*, an annual species known only from Cape York in Queensland. Some *Elionurus* species (e.g. *E. elegans* Kunth) have been characterised for the scent-producing volatiles. The Australian species *E. citreus* and *E. purpureus* have lemon-scented foliage and are known as Lemon Grasses or Lemon-scented Grasses.

In 2004, during a Department of Conservation and Land Management and Tropical Savannas Cooperative Research Centre survey in the King Leopold Ranges area of the Kimberley region of Western Australia (Start *et al.* 2012), an unusual grass was collected at one of the survey sites by T. Handasyde (TH). The specimen could not be confidently placed to genus, so it was sent to M. Lazarides in Canberra who suggested further material would be required to determine the genus. Subsequently, TH organised collection of further material from the same site in 2015 and 2017. Examination of both collections by M. Barrett (MB) confirmed that the material belonged to the genus *Elionurus*, possessing all the diagnostic characters of the genus as described above; however, the King Leopold specimens could not be accommodated in any known species (reviewed by Renvoise 1978 and Thompson 2017). It is therefore described below as a new species, *E. tylophorus* M.D.Barrett & T.Handasyde.

Methods

Morphology

Gross morphology of four accessions of *E. tylophorus* held at PERTH, including the proposed type, were examined in detail. Images of the types of *E. citreus* and *E. purpureus* were examined, as were all *E. citreus* collections held at PERTH and DNA.

DNA sequencing

In order to place the new species phylogenetically, DNA sequences were generated for two chloroplast regions, *ndh*F and *trn*K intron (including the *mat*K gene), and one nuclear rRNA region, the internal transcribed spacer (ITS), from the holotype specimen of *E. tylophorus*. All available *Elionurus* sequences from these regions were downloaded from GenBank, along with a range of outgroups from the tribe Andropogoneae (Table 1).

Extraction of genomic DNA was performed using a method modified from Doyle and Dickson (1987). The Carlson extraction buffer (Csaikl *et al.* 1998) contained 2% cetyltrimethylammonium bromide (CTAB), 1% polyethylene glycol (PEG), 1.4 M NaCl, 100 mM Tris-HCl and 20 mM EDTA (diluted from 0.5M pH 9.5 stock). Approximately 0.1 g of tissue was ground in 1 mL of buffer with 3 μ L of 100 mg/mL RNase (Qiagen, Melbourne, Victoria, Australia) added before being incubated at 65°C for 15 mins. Samples were then centrifuged at 11,500 rpm for 10 mins, and the supernatant transferred to a new tube, with 1 vol. of chloroform: isoamyl alcohol (24:1) added before being shaken for 30 mins. After centrifuging at 9,000 rpm for 10 mins, DNA was precipitated from the supernatant by adding 1 vol. of cold (-20°C) isopropanol to each sample and stored at -20°C in a freezer for 20 mins before pelleting the DNA in a centrifuge at 13,000 for 10 mins. The pellet was then washed in 500 μ L of 70% ethanol, re-centrifuged at 13,000 for 10 mins, and air-dried after removal of the ethanol.

Polymerase chain reactions (PCR) were performed on an Applied Biosystems Veriti® thermocycler, under the following conditions: 95°C for 3 mins, 37 cycles of ((95°C for 30 s, 48°C for 30 s, 72°C for 1.5 mins), 72°C for 10 mins). PCR amplifications were performed in 20 μ L volumes, and consisted of a final concentration of 67 mM Tris-HCl (pH 8.8 at 25°C), 16.6 mM (NH₄)₂SO₄, 0.45% Triton X-100, 0.2 mg/mL gelatin, 0.2 mM of each dNTP (all diluted 1:4 from 5× polymerisation buffer, Fischer Biotec, Subiaco, Western Australia), 2.5 mM MgCl₂, 0.12% v/v DMSO, 0.06% v/v BSA, and 0.2 units Taq DNA polymerase (Fischer Biotec, Subiaco, Western Australia), 0.5 μ M final concentration of each primer per reaction and *c*. 10–500 ng of template DNA. Primers used for DNA sequences were: ITS – 18SF / 26SR (Prince 2010); *mat*K – trnKmatKF / 2545R (Aliscioni *et al.* 2012); *ndh*F – 1311F / 2091R (Peterson *et al.* 2010).

Cycle sequence reactions were done using BigDye® Terminator v. 3.1 chemistry (Applied Biosystems) with the same primers as used for the PCR reaction, in both the forward and reverse directions, using the following PCR protocol: 96°C for 1 min, 25 cycles of (96°C for 10 s, 50°C for 5 s, 60°C for 4 min). Fragment visualisation was performed using an Applied Biosystems 3500 Genetic Analyser. Chromatograms were manually edited using CodonCode Aligner v.7.0.1 (CodonCode Corporation, Dedham, MA, USA, http://www.codoncode.com/). Comparisons to other sequences were made following alignment with sequences downloaded from GenBank (http://www.ncbi.nlm.nih.gov/) in the sequence database software Geneious® v.6.1.7 (http://www.geneious.com/; Kearse *et al.* 2012).

Phylogenetic analyses were performed separately for (1) a concatenation of *ndh*F and *trn*K intron (including the *mat*K gene) and (2) ITS, since the corresponding regions were only available for the same *Elionurus* sample in a few cases (Table 1). Outgroups were chosen to represent several different groups of Andropogoneae, since no close relatives of *Elionurus* have been identified in phylogeneis. Phylogenetic trees were reconstructed using RAxML (Stamatakis 2014), using a rapid bootstrapping and search algorithm, with 100 bootstrap replicates and a GTR+GAMMA nucleotide substitution model, as implemented in Geneious® v.6.1.7 (http://www.geneious.com/; Kearse *et al.* 2012).

Species	Voucher	ndhF	trnK / matK	ITS	Reference
Chrysopogon gryllus	<i>P.I. Kellogg</i> 250984 (A) (Rep. of Macedonia)	KY596161	KY596161ª		Arthan <i>et al.</i> (2017)
	O. Neamsuvan 165 (BCU)			GQ856347	Neamsuvan <i>et al.</i> (unpublished)
Elionurus citreus	S.J. & T.R. Hodkinson 9561 (TCD) (Australia)	HE573560	HE574449	GQ870207	Aliscioni <i>et al.</i> (2012); Teerawatananon <i>et al.</i> 2011
	K.R. McDonald KRM 14437 (BRI AQ914411) (Australia)			MK396908	This paper
	K.R. McDonald KRM 15855 (BRI AQ915512) (Australia)			MK396910	This paper
Elionurus muticus	<i>J.M. Kimeu et al.</i> JMK 145 (EA, K) (Kenya)	MF998637	MF998984		Hackel <i>et al.</i> (2018)
	Not specified			AF190758	Spies & Kellogg (unpublished)
Elionurus purpureus	P.I. Forster PIF 45151 (BRI AQ941416) (Australia)			MK396909	This paper
Elionurus royleanus	<i>M.S.</i> <i>Vorontsova</i> <i>et al.</i> MSV 826 (EA, K) (Kenya)	MF998646	MF998985		Hackel <i>et al.</i> (2018)
Elionurus tripsacoides	<i>Manrique</i> 1895 (COCA) (Mexico)			DQ005046	Skendzic <i>et al.</i> (2007)
Elionurus tripsacoides	<i>Manrique</i> 1904 (COCA) (Mexico)			DQ005047	Skendzic <i>et al.</i> (2007)

Table 1. Sequences used for phylogenetic analyses and GenBank numbers. Samples in bold were generated for this study.

Species	Voucher	ndhF	trnK / matK	ITS	Reference
Elionurus tristis	M.S. Vorontsova et al. MSV 589 (K, TAN) (Madagascar)	LN908091	LN906693		Hackel <i>et al.</i> (2018)
Elionurus tylophorus	<i>T. Sonneman</i> TH 8073 (PERTH, holotype) (Australia)	MK097174	MK097175	MK095935	This paper
Hemisorghum mekongense	<i>Traiperm</i> 569 (BKF) (Thailand)	KY596132	KY596132ª		Arthan <i>et al.</i> (2017)
Miscanthus transmorrisonensis	Not specified	LN869229	LN869229ª		Lloyd & Joshi (unpublished)
	Hodkinson 20 (K)			AY116271	Hodkinson <i>et al.</i> (2002)
Themeda villosa	<i>Arthan</i> 065, (BKF) (Thailand)	KY596131	KY596131ª		Arthan <i>et al.</i> (2017)
	E00697528			KY991079	Dunning <i>et al.</i> (2017)
	(China)				
Tripsacum dactyloides	Not specified (China)	NC_037087	NC_037087 ^a		Wang <i>et al.</i> (2017)
	<i>Sanchez-Ken</i> 607 (ISC) (Mexico)			DQ005086	Skendzic <i>et al.</i> (2007)

^a Extracted from a plastid genome, hence *ndh*F and *trnK / matK* have the same accession number.

Results

The chloroplast RAxML tree (Figure 1A) supported the monophyly of all sampled *Elionurus* species (100% bootstrap (BS) support). *Elionurus royleanus* A.Rich. was recovered as sister to all remaining species, but with low support (70% bootstrap, hereafter BS) support. Within the core *Elionurus* clade resolution was poor, and only a sister relationship between *E. muticus* (Spreng.) Kunth and *E. tristis* Hack. was supported (82%).

In the ITS RAxML tree (Figure 1B), *Elionurus* was not recovered as monophyletic due to the unresolved position of the single *E. muticus* sample. The *E. muticus* sample is very divergent from all other ITS sequences, and is likely a misidentified or contaminated sample, given that a different specimen of *E. muticus* is placed within the core *Elionurus* clade on chloroplast data. A clade containing all remaining *Elionurus* samples was strongly supported (100% BS), in the ITS tree, and divided into two sub-clades, one containing the two *E. tripsacoides* Kunth samples (100% BS), the other clade containing *E. tylophorus* sister to *E. citreus* (77% BS).



Figure 1. Phylogenies of *Elionurus* based on chloroplast and nuclear sequences. A – phylogenetic tree of ten chloroplast *ndh*F and *trn*K-intron sequences showing the monophyly of *Elionurus* and sister relationship of *E. royleanus* and all other *Elionurus* species; the position of two potential apomorphies for basal sister clades of *Elionurus* are indicated; B – phylogenetic tree of twelve ITS sequences showing the monophyly of *Elionurus*, a well-supported *E. citreus / E. purpureus* clade, and variation between *E. citreus* samples. See text for further discussion.

Discussion

Morphological comparison and evidence for a new species

A detailed summary of key features of the *Elionurus* collections are listed in Table 2. From this data, there are numerous discrete differences to both *E. citreus* and *E. purpureus*, spread across all parts of the plant. Thompson (2017) identified several apparently apomorphic features that unite the Australian species *E. citreus* and *E. purpureus*: pedicellate spikelets neuter (lacking anthers), and rachillas chartaceous, inflated and usually with a distal, bilobed flange. In comparison, all other *Elionurus* species, including *E. tylophorus* have male pedicellate spikelets and cartilaginous, slender rachillas lacking a flange. *Elionurus tylophorus* therefore is expected to lie outside of a presumed *E. citreus+E. purpureus* clade, but with unknown affinities to extra-Australian species.

Elionurus tylophorus can be accommodated in the Appendix 2 table of Thompson (2017) comparing all species of *Elionurus*, by listing the following character states: *paired spikelets* dissimilar; *sessile spikelet*: lower glume margin hairs pectinate (but the lower ones tubercle-based, resembling the tufted condition but with only a single apical hair); lower glume submargins with an oil streak; lower glume apex attenuate and 2-lobed; *pedicellate spikelet*: floret male; lower glume asymmetrical; lower glume margins with an oil streak on both margins; lower glume margins 2-keeled; lower glume apex long-attenuate, 1-lobed/awned. This combination of characters is unique, but most similar to *E. hensii* K.Schum. and *E. muticus. Elionurus hensii* shares with *E. tylophorus* the annual habit (according to Renvoise (1978), although Clayton *et al.* (2018) describe it as perennial), but differs in having smaller spikelets 3–4 mm long. *Elionurus muticus* is a perennial with short rhizomes and mostly basal leaves, and has spikelets in a pair similar to each other, while *E. tylophorus* is annual with cauline leaves and spikelets in a pair dissimilar.

The projections on the margins of the lower glume of the sessile spikelet of *E. tylophorus* are found in three other species, *E. elegans*, *E. hirtifolius* Hack. and *E. royleanus* (Renvoise 1978; Thompson 2017 Figure A3.1, A3.2), which might possibly indicate a relationship. However, these species all have tufts of hair on the submarginal projections of the sessile spikelet lower glumes, lack oil streaks on the glumes and have 1-keeled lower glume of the pedicellate spikelet. In contrast, *E. tylophorus* has submarginal projections of the sessile spikelet lower glumes at single hair, lower glumes of both sessile and pedicellate spikelets with two oil streaks, and lower glume of the pedicellate spikelet 2-keeled.

E. tylophorus therefore differs in multiple characters from all described species of the genus, indicating that species rank is appropriate for the new taxon. Keys are provided below to *Elionurus* in Australia, and to place *E. tylophorus* within the global key of Renvoise (1987). Different characteristics of *E. tylophorus* weakly suggest relationships with various other species in the genus, and true affinities will require molecular data.

Key to Australian species of Elionurus

1.	Keels (submarginal) of lower glume of sessile spikelet with a row of prominent projections often terminated by a single minute bristle, the projections grading into long cilia near glume apex; rachilla not winged at apex; pedicellate spikelet with lemmas and anthers	.E. tylophorus
1:	Keels (submarginal) of lower glumes of sessile spikelet pectinate (regularly ciliate), lacking projections; rachilla usually winged at apex; pedicellate spikelet lacking lemmas and anthers.	2

Elionurus tylophorus can be included in the global key to *Elionurus* species of Renvoise (1978) by adding the following couplet at the start of the key (see Thompson (2017) for the addition of *E. purpureus* to the Renvoise (1978) key):

1.	Keels (submarginal) of lower glume of fertile spikelet with a row of prominent projections, glabrous or terminated by a single minute bristle, the projections grading into long cilia near glume apex	E. tylophorus
1:	Keels (submarginal) of lower glumes of fertile spikelet with longer cilia either regularly arranged and lacking projections, or if with projections these terminated by hairs in tufts	Go to step 1 in Renvoise (1978)

 Table 2. Distinguishing morphological characters between the three Australian *Elionurus* species (after Thompson 2017).

Character	Elionurus citreus	Elionurus purpureus	Elionurus tylophorus
Growth habit	perennial (or occasionally annual according to collector notes and herbarium specimens)	annual	annual
Inflorescence type	single racemes at nodes on cultivated plant and topotypes	multiple racemes at nodes at least on cultivated plants	multiple racemes at nodes on wild-collected plants
Culms (shape in T.S.)	narrowly convexo- concave	broadly convexo-concave	broadly convexo-concave
Leaf blades and sheath			
Distribution	mostly basal	cauline	cauline
Margin prickle hairs	medium (60-70 um)	absent	present, to 1.5 mm long
Margins and mid-vein indumentum	usually scabrous	smooth	smooth
Mid-vein	acutely keeled	obtusely keeled	±obtusely keeled
Sessile spikelet (mid-raceme)			
Total length (beak, body and lobes) (mm)	10.6–12.1	8.1–9.2	9.0–13.2
Colour at maturity	pallid to pale pink, rarely purplish	purple	green turning straw with purplish margins or pale purplish all over
Lower glume			
lobe length (mm)	5.5–7	2.6-3.2	2.0-4.5

Character	Elionurus citreus	Elionurus purpureus	Elionurus tylophorus
body width \times length (mm)	1.5–1.7 × 3.6–4.3	1.7–2.0 × 3.5–4.2	1.4–2.0 × 7.0–12.0
texture	cartilaginous	chartaceous	cartilaginous
nerve prominence	not raised	not raised	strongly raised
indumentum type	glabrous to pilose	pubescent	pubescent in rows between nerves
orientation	ascending	appressed	ascending (appressed)
hair length (mm)	<i>c</i> . 2	<i>c</i> . 0.2	0.05-0.25
keeled margins	narrowly winged	not winged	very narrowly winged
Upper lemma			
margin texture	hyaline	hyaline	pale straw
marginal indumentum	ciliate apically or glabrous	pilose	±sparely ciliate
apex	attenuate	acute	attenuate
Callus length (mm)	1.8–2.7	1.0–2.3	1.5-2.5
Pedicellate spikelet			
Lower glume			
width \times length (mm)	$c. 0.9 \times 7.2 - 8.5$	$0.8 - 1.3 \times 5.2 - 5.7$	$0.8 - 1.2 \times 9 - 11.7$
colour at maturity	pallid to pale pink, rarely purplish	purple	green turning straw with purplish margins or pale purplish all over
body	linear to narrow lanceolate	lanceolate	lanceolate to narrowly lanceolate
	asymmetrical	asymmetrical	asymmetrical
	3-veined	5-veined	7-veined
	1-keeled	1-keeled	2-keeled
	one margin with oil streak	both margins with oil streak	both margins with oil- streak
Upper glume	unequal to lower glume	subequal to lower glume	subequal to lower glume
veins	3-veined	5-veined	obscurely 3-5-nerved
shape in section	laterally compressed	rounded	rounded to laterally compressed
Lemmas	absent	absent	present
Anthers	absent	absent	present
Anther length			
Chasmogamous (mm)	1.3–2.7	0.9–2.0	2.5-3.0
Cleistogamous (mm)	0.7-1.8	<i>c</i> . 0.8	not seen
Pedicellate floret	absent	absent	2.2–3.8
Rachilla			
Width \times length (mm)	$0.9-1.3 \times 3.3-5$	$1.2-1.4 \times 4.3-4.4$	$0.9-1.2 \times 7.5-16$,
Shape	clavate	distinctly clavate	parallel-sided

Character	Elionurus citreus	Elionurus purpureus	Elionurus tylophorus
Apex rim	usually narrowly winged and unequally bilobed, or not winged	distinctly winged, unequally bilobed	entire, not winged, not bilobed
Subapical beard length of longest hairs (mm) Pedicel	3.6-4.9	2.8–2.9	2.5-4.5
Width \times length (mm)	$0.5-0.6 \times 2.9-4.0$	$c. 0.8 \times 2.5 - 3.5$	0.6–0.8 × 4.1–6.2

Phylogeny of Elionurus

In the chloroplast ndhF + trnK tree (Figure 1A), *E. royleanus* was found sister (70% BS) to all other *Elionurus* species, including *E. tylophorus. Elionurus royleanus* was the only sampled representative of three similar species with tufted hairs on the submargins of the lower glume of the sessile spikelet (the others being *E. elegans* and *E. hirtifolius*, hereafter the 'tufted group'). These unique hair-tufts, along with other similarities (see Appendix 2 in Thompson 2017) suggest that the 'tufted group' might be a monophyletic clade sister to the remaining 'core group' of *Elionurus*; if so the presence of 1 or 2 oil streaks on the lower glumes would then be an apomorphy for the 'core group' (oil streaks are lacking in the 'tufted group'; Thompson 2017). The presence of glume hair tufts and oil streaks might then be reciprocal apomorphies for a primary dichotomy within *Elionurus*. The position of *E. tylophorus* remote from *E. royleanus* suggests that the tuberculate projections on lateral submargins of the lower glume of the sessile spikelet found in the 'tufted group' and *E. tylophorus* are not indicative of a close relationship, but instead arose through convergence or homoplasy. The relationship between *E. tylophorus* and *E. citreus* was insufficiently resolved, and too many potentially intermediate species remain unsampled, to draw any conclusions about the monophyly or otherwise of Australian *Elionurus* from the chloroplast data.

In the ITS tree (Figure 1B), the available samples of *Elionurus* are supported (90% BS) as a monophyletic group, however no species of the 'tufted group' discussed above have ITS sequences available. A clade composed of the three representatives of E. citreus and one E. purpureus was strongly supported (90% BS), to the exclusion of E. tylophorus. This E. citreus clade is in agreement with the morphological evidence presented in Thompson (2017) for a close relationship between E. citreus and E. purpureus, and also corroborates the distinctiveness of E. tylophorus from the other Australian species. Two of the E. citreus specimens (K.R. McDonald 15855 and S.J. & T.R. Hodkinson 9561) formed a strongly supported clade (95% BS) and were divergent from the third specimen of E. citreus (K.R. McDonald 14437), which formed a polytomy with the sample of E. purpureus. This result suggests that E. citreus might not be monophyletic with the separation of E. purpureus. The specimens K.R. McDonald 15855 and K.R. McDonald 14437 had been annotated as belonging to 'small spikelet' and 'long spikelet' forms respectively, which could indicate a morphological difference between them. The ITS data therefore argue for further taxonomic revision of E. citreus across its range. Although a sister relationship was recovered between the E. citreus – E. purpureus clade and E. tylophorus, it was poorly supported (58% BS). Since *E. tripsacoides* is the only non-Australian species for which ITS sequences are available, it is not yet possible to draw any conclusions about the monophyly or otherwise of the Australian species. The ITS sequence generated for E. tylophorus differs in 18-21 substitutions plus several indels (95% similarity) from the sequences of *E. citreus*, which is considerably greater than is typically observed between closely related grass species (only 3 or 4 substitutions between E. purpureus and the various E. citreus sequences). The number of substitutions between E. tylophorus and E. citreus is comparable

to the difference between *E. tylophorus* and a Mexican accession of *E. tripsacoides* (DQ005047, Skendzic *et al.* 2007; 20 substitutions plus several indels, 94% similarity). The Australian *E. citreus* – *E. purpureus* clade and *E. tylophorus* therefore seem to be relatively distantly related to each other. Greater sampling of ITS and other loci within *Elionurus* will be required to determine whether the two Australian lineages arose following independent dispersals into Australia or represent a single more ancient lineage that speciated within Australia.

The apparent restricted distribution of *E. tylophorus* is puzzling, given that its habitat is unassuming, appears representative of a widespread soil type and plant community, and is not obviously linked to any localised geological or hydrological feature (Figure 2). Many grass species in the Australian Monsoon Tropics have wide distributions, often extending to south-west Asia or beyond; however, occasional species with apparently restricted distributions do exist (e.g. *Clausospicula extensa*, a restricted monotypic genus in the Top End, Lazarides *et al.* 1991). It is possible that *E. tylophorus* has simply been overlooked amongst the array of other co-occurring grasses and is in fact more widespread than current data suggests.

Taxonomy

Elionurus tylophorus M.D.Barrett & T.Handasyde, sp. nov.

Type: King Leopold Ranges, Western Australia [precise locality withheld for conservation reasons], 13 April 2015, *T. Sonneman* TH 8073 (*holo*: PERTH 08662908; *iso*: BRI, K, PERTH 09033955 (smutted specimen)).

Erect annual grass 45–60 cm tall; culms <4 mm wide, unbranched, green to reddish; nodes 4–8, glabrous, *internodes* purplish, ±terete near base, broadly convexo-concave (±D-shaped) above, mostly glabrous but just below inflorescence-subtending node with appressed hairs along the angles, hairs becoming longer (up to 4.5 mm long) just below the node. *Ligule* a dense fringe of hairs 0.7–2.5 mm long. Leaves cauline; leaf sheath 26–52 mm long, 9–23-nerved, glabrous or sparsely hispid near apex; leaf blades linear, flat to canaliculate or involute, 65–175 mm long, 0.7–2.5 mm wide, 9–11-nerved, \pm rounded on the abaxial surface but with raised nerves that are sometimes \pm acute, adaxial surface glabrous to sparsely hispid near the base, abaxial surface glabrous except for the submargins with sparse tubercles often terminated by a stiff hair up to 1.5 mm long. Inflorescence a spatheolate raceme but appearance spiciform, racemes in upper 1–3 nodes. Spatheoles 45–69 mm long, mostly tightly sheathing and obscure, with a small, erect blade enclosing only the base or sometimes to apex of the lowest spikelet at maturity. *Racemes* 1–7 per node, 35–75 mm long, 3–8 mm wide, of 4–12 spikeletpairs; rachis fragmenting at maturity, rachillas free from the internode, dissimilar to pedicel, shorter than or longer than pedicels, $7.5-16.0 \text{ mm} \log (\text{tip to tip}), 0.9-1.2 \text{ mm} \text{ thick at widest point, \pm parallel$ sided, hollow, oblique at joints leaving sharply acute apices, somewhat flattened in section, mostly glabrous to very sparsely minutely to shortly hairy, but abaxial edge densely shortly hairy in a broad band with hairs ascending, 0.7–1.0 mm long, with longer stiff white hairs at apex, the longest hairs 3.0–4.5 mm long; apex oblique, not winged or bilobed; disarticulation scar flattened, rim thickened, glabrous. Spikelets paired, one sessile and fertile, the other pedicellate and sterile, dimorphic within the pair, not gradational (similar length along the raceme), appressed and closely overlapping in two opposed staggered rows along a jointed disarticulating axis; diaspore composed of a pair of spikelets remaining attached to the rachilla. Sessile spikelet containing 2 florets, the lower sterile, the upper bisexual, apparently always chasmogamous, 9.0-13.2 mm long (including callus and proximal beak), 1.4–2.0 mm wide, dorsiventrally compressed; proximal beak c. 1.4–1.8 mm long, glabrous or hairy along margins and gradually merging with callus; callus elliptic, c. 1.0-1.5 mm long, gradually merging with beak, apex moderately acute to obtuse in face view, sharply acute in side view, glabrous along broad midline, pubescent with white hairs to 0.8–1.2 mm long on lateral faces, green turning straw sometimes with purplish margins or pale purplish all over; glumes dissimilar; lower glume of sessile spikelet 7-12 mm long (excluding callus), 1.4-2.0 mm wide, oblong to ±elliptic, broadest near the middle, apex bifid with 2 long beak-like teeth each 2.0-4.5 mm long (much shorter than glume body), body thickly indurated, prominently 6–8-veined with thickened raised veins, strongly 2-keeled (keels thickened, sub-marginal and highly divergent, with nerves between them), body with sparse \pm appressed to pubescent white hairs 0.05–0.25 mm long in lines in the narrow grooves between nerves, the grooves narrower than the nerves, a narrow, pale reddish brown oil streak present just inside each of the keels in the basal half (extending onto the inner face); keel not winged, with a row of strong tuberculate projections 0.15-0.3 mm tall in the lower 1/2-4/5 of the body, projections rounded to acute containing a single oblique apical hair up to 0.5 mm long, gradually merging into a dense fringe of stiff white hairs on keel margin (the longest hairs 0.5-0.8 mm long); glume margins very narrow, ±membranous, entire, slightly in-rolled beyond the keels and difficult to observe; upper glume of sessile spikelet 7–8 mm long, 1.7–2.0 mm wide, narrowly 1-keeled (carinate), 3-veined, shortly acuminate, chartaceous to coriaceous in the inner part between the nerves, membranous to chartaceous marginally outside the lateral nerves, shortly hairy (0.05–0.4 mm, irregular orientation, ascending) in a band either side of the keel and including on the keel, glabrous on marginal areas, narrow membranous margins with sparse to moderately sparse cilia (0.2–0.4 mm, wavy) in upper half; lower lemma of sessile spikelet 4.2–4.7 mm long, c. 1.4 mm wide, lanceolate, acute, lacking nerves or obscurely 2-4-nerved near base, membranous, pale straw to pale brown near base, sub-hyaline above and on margins, glabrous or minutely ciliate on upper margins, upper lemma of sessile spikelet elliptic, 4.2–4.6 mm long, c. 1.3 mm wide, lanceolate, acute to acuminate, membranous to chartaceous, pale straw to sub-hyaline, 0-nerved or obscurely to moderately 3-nerved, glabrous or with a few cilia c. 0.25 mm long in upper part; lower and upper paleas of sessile spikelet absent; anthers 3, c. 2.5-3.0 mm long, purple; styles 2, exserted at anthesis, purple; caryopsis 3.6–4.9 mm long, 1.0–1.1 mm wide, moderately to distinctly dorsiventrally compressed, hilum half as long as caryopsis; lodicules 2, c. 0.4 mm long, membranous, broadly rounded at apex, entire. Pedicellate spikelet 8.0-11.7 mm long (excluding pedicels), 2–8 mm wide, rounded to weakly dorsiventrally compressed, with two florets, the lower sterile and the upper functionally male (anther may be difficult to demonstrate in older specimens with dropped anthers) with 2 glumes and 2 lemmas, initially lanceolate but gaping at maturity; pedicels 4.1-6.2 mm long, 0.6-0.8 mm wide, glabrous on one face, the opposite 'external' face with a longitudinal curving arc of short hairs that terminates in a sub-apical tuft of hairs 2.5-4.5 mm long; glumes dissimilar; lower glume of pedicellate spikelet asymmetric most noticeable in keel indumentum, 9.0–11.7 mm long, 0.8–1.2 mm wide, dorsiventrally compressed, lanceolate to narrowly lanceolate, apex long-acuminate into an awn (2.0-)4.0-6.0 mm long, coriaceous, green to pale purplish all over, obscurely to moderately strongly 7-veined (not or scarcely raised, visible in transmitted light), 2-keeled close to margins, oil-streak present on both sides, with the keel glabrous or with stiff white hairs c. 0.2 mm long (indumentum usually asymmetric, more prominent on one keel than the other), margins membranous, 0.2–0.4 mm wide and wider on one side than the other; upper glume of pedicellate spikelet rounded to laterally compressed, shorter, subequal to or slightly shorter or longer than lower glume, very narrowly lanceolate, long-attenuate into an awn c. 3-5 mm long, indurated in central zone, chartaceous to membranous on sides, 1-keeled ±in centre, obscurely to moderately 3-5-nerved, scabrous along upper keel and margin, glabrous; lower lemmas of pedicellate spikelet 3.0–4.0 mm long, 0.8–0.9 mm wide, lanceolate, acute to muticous, chartaceous to membranous, not nerved, glabrous or with marginal cilia in upper part; upper lemma of pedicellate spikelet shorter than lower, 2.6-3.0 mm long, glabrous, otherwise similar to lower lemma; paleas absent; anthers present in upper floret, 3, apparently dimorphic, 2.2-3.8 mm long and exserted at maturity (a few 0.4 mm long are probably merely immature), purple; ovary and styles rudimentary, c. 0.2 mm long, not developing further. (Figure 3)

Diagnostic features. Elionurus tylophorus differs from all known *Elionurus* species in having keels (that are submarginal) of the lower fertile glumes ornamented with projections, which are often terminated by minute solitary bristles. Other notable features include: annual habit, prominently thickened lower glume nerves in the fertile (sessile) floret, pedicellate spikelets male with well-developed lemmas and anthers, lower glume body \pm oblong with parallel-sided lateral outline, and glumes of the pedicellate spikelet subequal in length and awned.

Other specimens examined. WESTERN AUSTRALIA: [localities withheld for conservation reasons] same locality as type, 29 May 2017, *K. Carnes* TH 8193 (DNA, KNR, PERTH); same locality as type, 26 May 2004, *T. Handasyde* TH 2148 (BRI, CANB, DNA, KNR, PERTH); same locality as type, 13 April 2015, *T. Sonneman* TH 8072 (PERTH).

Phenology. Fertile collections have been made between April and May (the latter senescing).

Distribution and habitat. The single known *E. tylophorus* population was growing on a rocky ridge/slope of colluvium and alluvium in a sparse low woodland of a mix of species including *Eucalyptus tectifica*, *E. brevifolia*, *Corymbia cadophora* and *Erythrophleum chlorostachys* over scattered *Cochlospermum fraseri* and *Calytrix achaeta* (Figure 2). Ground cover included sparse to moderately dense *Heteropogon contortus*, *Eriachne sulcata*, *Mnesithea rottboellioides* and *Cymbopogon bombycinus*. *Elionurus tylophorus* is currently known from a single location in a geologically diverse section of the King Leopold Ranges in the central Kimberley.



Figure 2. Habitat of Elionurus tylophorus from type location. Arrow indicates E. tylophorus, drying straw-coloured at time of photo.



Figure 3. *Elionurus tylophorus (T. Sonneman* TH8073). A – splayed spikelet pair, showing pedicellate spikelet, fertile sessile spikelet and rachis internode; B – abaxial surface of base of lower glume of sessile spikelet showing strongly raised nerves, short hairs in rows between nerves, and marginal projections; C – adaxial surface of base of lower glume of sessile spikelet showing projections, and upper glume showing keel and surface hairs; D – dissected spikelet pair: a – rachilla, b – male pedicellate spikelet with lemmas visible, c – lower glume (with callus cut away), d – upper glume, e – lower lemma, f – upper lemma, g – caryopsis, h (insert) – apex of rachilla, which lacks a wing and is not bilobed. All scale bars 1 mm.

Conservation status. To be listed as Priority Two under Conservation Codes for Western Australian Flora (M. Smith, pers. comm.). It is conserved in the King Leopold Ranges Conservation Park (previously Mt Hart Station).

Etymology. The species epithet is Greek from *tylo*- (with knobs, lumps or projections), and *-phorus* (-bearing).

Notes. Cleistogamous flowers have been reported in both of the other Australian species, *E. citreus* and *E. purpureus* (Thompson 2017), but were not detected in any of the three specimens of *E. tylophorus*. However, plants were not cultivated, and some of the available *E. tylophorus* material is over-mature. In the *K. Carnes* TH 8193 collection, the best racemes have styles that are exserted but have already exserted and dropped their anthers from all sessile florets, while the sterile florets all have exserted or dropped anthers. In this collection at least, it appears that cleistogamous flowers are lacking, the sessile flowers are protandrous (as in most grasses), while the styles of sessile spikelets are \pm synchronous within a raceme and with anthers of the pedicellate spikelets.

Racemes in *Elionurus* fragment from the apex downward; although the lowermost spikelet is the last to fall in *E. tylophorus*, it does not appear tardily dehiscent as reported for *E. purpureus* (and at least one *E. citreus* plant) by Thompson (2017). Much of the available material is in the stage of dehiscing, and even prior to the secondmost-basal spikelet dehiscing the basal joint is already weak, suggesting that there is no significant delay of dehiscence of the basal florets.

The characteristic marginal projections on the lower fertile glumes of *E. tylophorus* are possibly developmentally homologous with tuberculate bases of hairs, as found on leaves in the same species, since the projection is frequently terminated by a single short hair. Alternatively, since the projections are tightly fused with the keel, they may be outgrowths of the keel itself, centred on hair-cells.

Elionurus tylophorus differs from all known *Elionurus* species (Renvoise 1978) in having submarginal projections on the keels of the lower fertile glumes, which are often terminated by minute solitary bristles, compared to cilia or hair-tufts that are not swollen at the base in other species. *Elionurus tylophorus* is also unusual within the genus in its annual habit. Aside from *E. purpureus* discussed above, the only other annuals or short-lived perennials in the genus are the African and Indian species *E. elegans, E. hensii* and *E. royleanus* (Renvoise 1978).

Elionurus tylophorus differs further from both of the only other Australian *Elionurus*, *E. citreus* (Figure 4) and *E. purpureus* (Thompson 2017), in its annual habit (usually tufted perennial in *E. citreus*), prominent thickened lower glume nerves in the fertile (sessile) floret (lower glume smooth or with thin scarcely raised nerves in *E. citreus*), lemmas and anthers present in pedicellate spikelets (absent in *E. citreus* and *E. purpureus*), lower glume body \pm oblong with parallel-sided lateral outline (ovate and rounded lateral outline in *E. citreus* and *E. purpureus*), and glumes of the sterile spikelet roughly subequal in length (upper glume usually 1/2-2/3 as long as the lower glume in *E. citreus* and *E. purpureus*). Further differences between all three Australian species are contrasted in Table 2, and in the keys.

The leaves of *E. citreus* have a lemon scent when crushed, but only a weak scent from crushed inflorescences is reported for *E. tylophorus* (*K. Carnes* TH 8193).



Figure 4. *Elionurus citreus* (*G.N. Batianoff* 10118, PERTH 01780565, QLD, Lizard Is.), which is a good match for the holotype of *E. citreus* [*Andropogon citreus* R.Br.; *R. Brown* 6176, 1802; images of holotype at K and isotypes at E, W seen], except in being less obviously perennial than the type. A – splayed spikelet pair, showing sterile pedicellate spikelet, sessile spikelet and rachilla with expanded apex; B – base of lower glume showing obscure nerves, and marginal cilia (rachilla and pedicel cut away from callus); D – dissected spikelet pair: a – rachilla showing slightly winged and bilobed apex, b – sterile pedicellate spikelet, c – lower glume (with callus cut away), d – upper glume, e – lower lemma, f – upper lemma. All scale bars 1 mm.

Elionurus tylophorus superficially resembles some slender species of *Ischaemum* L. or *Sehima* Forssk., but is immediately distinguished by lacking an awn on the fertile lemma (twisted and geniculate awn present in *Ischaemum* and *Sehima*).

Two of the collections are smutted [K. Carnes TH 8193 (PERTH 08654972) and TH 8073 (PERTH 09033955)]. No smut has previously been reported on *Elionurus* from Australia (Shivas *et al.* 2014), but three species, *Anthracocystis elionuri* (Henn. & A.Evans) McTaggart & R.G.Shivas, *Macalpinomyces elionuri-tripsacoidis* Vánky and *Sporisorium elionuri-tristis* Vánky have been described from *Elionurus* in Africa (Vánky 1999, Vánky 2003; McTaggart *et al.* 2012a, b). Future work may show that the smut on *E. tylophorus* is an undescribed species, and if so may be rare along with its host.

The vernacular name Toothed Wire Grass is suggested.

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